

CLAIMS

1. A method for clonal derivation of human blastocyst-derived stem cells (hBS) or hBS derived cells, the method comprising the steps of

- 5 a) subjecting hBS cell colonies or hBS derived cell colonies to non-enzymatic treatment to dissociate the cell colonies to one or more single cells,
b) selecting/picking of one or more single cells,
c) separately cultivating the one or more single cells in a serum based medium and/or serum based conditioned medium,
10 d) optionally, changing the medium to a serum free medium to obtain one or more cell clones capable of forming colonies.

2. A method according to claim 1, wherein the steps a)-d) are preceded one or more times by the following steps

- 15 a1) subjecting hBS cell colonies or hBS derived cell colonies to non-enzymatic treatment to dissociate the cell colonies to substantially single cells,
b1) selecting/picking of one or more substantially single cells,
c1) separately cultivating the one or more substantially single cells in a serum based medium and/or serum based conditioned medium,
20 d1) optionally, changing the medium to a serum free medium to obtain a substantially pure cell population.

3. A method according to claim 1 or 2, wherein the non-enzymatic method comprises the steps of

- 25 i) cutting hBS cell colonies or hBS derived cell colonies to obtain smaller units.
ii) incubating the smaller units with a medium containing a chelator such as, e.g., EDTA,
iii) triturating the smaller units to obtain hBS single cells or hBS derived single cells.

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4. A method according to claim 3, further comprising a step of

- iv) dispersing the hBS single cells or hBS derived single cells in a suitable medium, such as, e.g. a cell free hBS cell conditioned cloning medium (CC-medium), a hBS derived cell free conditioned medium, serum based medium or a hBS
35 culture medium.

5. A method according to any of the preceding claims, wherein step d) and/or step d1) is included.
6. A method according to any of the preceding claims, wherein the cell clones obtained in step c) and/or step d) are further cultivated.
7. A method according to any of the preceding claims, wherein the substantially pure cell populations obtained in step c1) and/or step d1) are further cultivated.
8. A method according to any of the preceding claims, wherein the obtained cell clones are hBS cell clones.
9. A method according to any of the preceding claims, wherein the hBS derived cells are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.
10. A method according to any of the preceding claims, wherein the obtained cell clones are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.
11. A method according to any of the preceding claims, wherein the hBS derived cells are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.
12. A method according to any of the preceding claims, wherein the obtained cell clones are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.
13. A method according to any of the preceding claims, wherein the cultivation in step c) and/or c1) is performed in a medium that promotes propagation of the one or more hBS cells or hBS derived cells.
14. A method according to any of the preceding claims, wherein step c) and/or step c1) is performed in the presence of fibroblasts, such as, e.g. mouse embryonic fibroblasts, human foreskin fibroblasts, fetal skin fibroblasts, fetal muscle fibroblasts, adult skin

fibroblasts and fibroblasts derived from hBS cells.

15. A method according to any of claims 1-13, wherein step c) and/or step c1) is performed under feeder cell free conditions.

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16. A method according to claim 15, wherein step c) and/or step c1) is performed on a support substrate comprising a component that promotes colony formation and/or cell division and/or adhesion and/or inhibits differentiation of the hBS single cells or hBS derived single cells, such as, e.g. albumin, gelatine, poly-ornithine, fibronectin,

10 vitronectin, agarose, poly-L-lysine, collagen, and/or extracellular matrix components, such as, e.g. Matrigel® or laminin and/or combinations thereof.